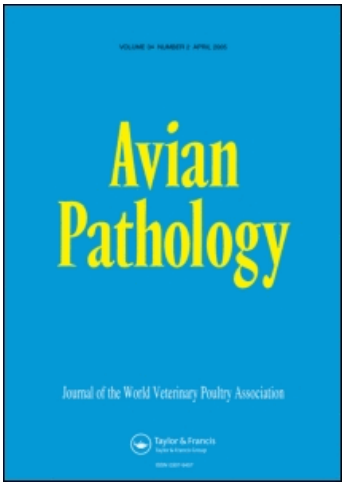

Avian Pathology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713405810

Retrospective evidence that the MHC (B haplotype) of chickens influences genetic resistance to attenuated infectious bronchitis vaccine strains in chickens

L. D. Bacon [a]; D. B. Hunter [b]; H. M. Zhang [a]; K. Brand [c];R. Etches [c]
[a] US Department of Agriculture, Agricultural Research Service, Avian Disease and Oncology Laboratory, East Lansing, Michigan, USA [b] Department of Pathobiology, Ontario Veterinary College, Guelph, Ontario, Canada [c] Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada

To cite this Article Bacon, L. D. , Hunter, D. B. , Zhang, H. M. , Brand, K. andEtches, R.(2004) 'Retrospective evidence that the MHC (B haplotype) of chickens influences genetic resistance to attenuated infectious bronchitis vaccine strains in chickens', Avian Pathology, 33: 6, 605 — 609
To link to this Article: DOI: 10.1080/03079450400013147
URL: http://dx.doi.org/10.1080/03079450400013147

Avian Pathology (December 2004) **33(6),** 605–609

Taylor & Francis
Taylor & Francis Group

Retrospective evidence that the MHC (B haplotype) of chickens influences genetic resistance to attenuated infectious bronchitis vaccine strains in chickens

L. D. Bacon[1]*, D. B. Hunter[2], H. M. Zhang[1], K. Brand[3†] and R. Etches[3‡]

[1]US Department of Agriculture, Agricultural Research Service, Avian Disease and Oncology Laboratory, East Lansing, Michigan 48823, USA, [2]Department of Pathobiology, Ontario Veterinary College, and [3]Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

Infectious bronchitis is a respiratory disease of chickens that is caused by the coronavirus infectious bronchitis virus (IBV). Virtually all broiler and layer breeder flocks are routinely vaccinated against IBV. Two hatches of 1-day-old chicks from four lines were mistakenly vaccinated for infectious bronchitis using a moderately attenuated vaccine designed for chicks of an older age. The vaccination resulted in high mortality, and chicks from three of four lines died with signs typical of infectious bronchitis. The mortality that occurred using this less-attenuated vaccine was significantly influenced by the genetic line, and the MHC (B) haplotype in chickens of three B congenic lines. B congenic chickens possessing the *B*15* haplotype were resistant in contrast to chickens possessing the *B*13* or *B*21* haplotypes. Chicks from two further hatches of the four lines were vaccinated appropriately with a more attenuated IBV vaccine, and only limited chick mortality was seen. These retrospective data from two repeated hatches confirm earlier data indicating chicken genes influence resistance to IBV, and indicate for the first time that genes tightly linked to the *B* haplotype are relevant in resistance to IBV. Due to extenuating circumstances it was not possible to verify results with chicks from F_2 matings. Factors that may enhance definition of the role of the *B* haplotype in immune response to IBV, and the desirability for further analysis of a *B* haplotype-linked influence on immunity to IBV are discussed.

Introduction

Infectious bronchitis virus (IBV) is a member of the *Coronaviridae* in the *Coronavirus* genus. It causes a respiratory disease of chickens known as infectious bronchitis (IB) (Raj & Jones, 1997; Cavanagh & Naqi, 2003). Virtually all broiler breeder and layer breeder flocks are routinely vaccinated against IBV, and in many areas broilers are also vaccinated. The standard vaccination program for breeder birds would be at least two vaccinations during the growing period using modified live products to activate the immune system. The initial vaccine would contain a milder (more attenuated) vaccine strain, and the second would utilize a less attenuated strain. Then an oil immulsion killed vaccine is administered just prior to the birds entering the laying period. This immunization of chickens with modified live vaccines maximizes the bird's immune response to the killed product, with the aim of having solid immunity extending throughout the period of egg production.

Herein we describe the results of mistakenly vaccinating two hatches of 1-day-old chicks for IB using a less attenuated vaccine designed for chicks of an older age. This vaccination resulted in

*To whom correspondence should be addressed. Tel: +1 517 337-6831. Fax: +1 517 337-6776. E-mail: baconld@msu.edu
† Current address: 765 Fraser Street, Sudbury, Ontario, Canada, P3E 2B3.
‡ Current address: Origen Therapeutics, 1450 Rollins Road, Burlingame, California 94010, USA.
Received 4 May 2004. Accepted 16 August 2004
ISSN 0307-9457 (print)/ISSN 1465-3338 (online)/04/06605-05 © 2004 Houghton Trust Ltd
DOI: 10.1080/03079450400013147

high mortality in three lines of chickens, whereas little mortality was seen in one line. Interestingly, the mortality caused by misusing this less-attenuated vaccine significantly differed among genetic lines, and among MHC congenic lines. Chicks from two further hatches of the five lines were vaccinated with a more attenuated IBV vaccine, and only limited chick mortality was seen.

Materials and Methods

Chicken lines. Fertile eggs were obtained from specific pathogen free breeders of inbred lines 7_1 and $15I_5$, and two MHC (B) congenic lines developed in line $15I_5$ (Bacon *et al*., 2000). Line $15I_5$ and the two B congenic lines are 99.9% identical except that each contains a unique MHC haplotype. The parental inbred line $15I_5$ has B15, line 15.P-13 has B13 and line 15.N-21 has B21. The relative break-points for the B loci in regards to the rest of chromosome 16 in the B congenic lines is undefined, although they all possess the same RfpY genotype. The breeders had only received one vaccine (i.e. herpesvirus of turkeys for Marek's disease [MD]), and therefore the chicks lacked maternal antibodies to IBV. Each hatch of chicks was placed in a separate isolation room at the Arkell Poultry Research Station, University of Guelph. Importantly, the chicks from all lines were intermingled and grown for 60 days on the floor using wood shavings as litter. At 1 day of age chicks were vaccinated as described in the following. All mortality was documented and summarized for all chicks for 60 days. Necropsies were carried out on several chicks that died from the first two hatches and gross lesions were recorded. The lung, trachea and other organs were fixed in 10% buffered formalin for 48 h, imbedded in paraffin, sectioned at 6 mU, stained with hematoxylin and eosin, and examined by light microscope. The lung and trachea were sent for virus culture.

Vaccinations. At 1 day of age all chicks received a subcutaneous vaccination against MD using Live Turkey Herpesvirus (Solvay Animal Health Inc., Kitchener, Ontario, Canada) as directed. The newly hatched chicks were also administered IB vaccines by eye-droplet according to instructions. Hatches one and two were mistakenly vaccinated with vaccine A. This vaccine contained the Holland strain of Massachusetts IBV. It is intended for use in chicks 4 weeks of age or older that have been previously vaccinated with a milder vaccine. It is not recommended for initial vaccination. When it was realized the incorrect vaccine had been used, hatches three and four were vaccinated according to the standard schedule with vaccine B that contained a cloned Massachusetts (Ma5) strain of IBV. This mild vaccine is intended for use in bird's 1 day of age or older.

Statistical analysis. Chicks from hatches one and two were vaccinated with a lowly attenuated IBV vaccine whereas chicks from hatches three and four were vaccinated with a highly attenuated vaccine. Within each type of vaccine the percentage of death and days to death for the combined data of two hatches were analyzed. The percentage of death between lines was compared with an adjusted chi-square test (Geng & Hills, 1989). The days to death among the lines was analyzed with a one-way analysis of variance. The differences of average days to death between lines were compared with a Duncan's multiple-range test (SAS Institute, 2001).

Results

Table 1 summarizes the number of chicks, the percent mortality, and the mean age to death of chicks in each line in each hatch for 60 days. In the first two hatches about two-thirds of the chicks in line 7_1 died. Moreover, nearly 50% of the chicks died in the B congenic lines 15.P-*13* and 15.N-*21*. In marked contrast, only 12% of the $15I_5$ chicks died. These differences in mortality ($P<0.01$) were seen repeatedly in each hatch. The chicks in lines 15.P-*13* and 15.N-*21* died at a younger age than chicks of line $15I_5$ ($P<0.01$). Many of the dying chicks were gasping, and sample chicks were necropsied. Macroscopic changes observed at necropsy included hyperemia of the tracheal mucosa and yellow–white exudates within the tracheal lumen. In some birds tracheal plugs were present within the tracheal lumen, and in others the laryngeal opening was completely occluded with exudate (Figures 1 and 2). The air sacs of some birds were moderately thickened and small amounts of yellow debris and fibrin was present on the air sac surfaces. Microscopic lesions were most severe in the trachea and included hyperemia

Table 1. *Mortality caused by lowly and highly attenuated IB vaccines*

	Lowly attenuated IB vaccine				Highly attenuated IB vaccine			
Line	Hatch	Number of chicks	% dead[a]	Days to death (mean ± standard error)[b]	Hatch	Number of chicks	% dead[a]	Days to death (mean ± standard error)[b]
$15I_5$	1	27	7^{A}	14.0 ± 2.0	3	20	0^{AB}	N/A
	2	40	15^{A}	19.0 ± 4.5^{B}	4	None	–	–
	Both	67	12^{A}	17.7 ± 3.4^{B}	Both	20	0^{AB}	N/A
15.P-*13*	1	34	50^{B}	10.7 ± 1.8	3	43	9^{B}	7.8 ± 0.5^{A}
	2	32	44^{B}	8.4 ± 0.9^{A}	4	17	12^{B}	12.5 ± 0.5
	Both	66	47^{B}	9.6 ± 1.1^{A}	Both	60	10^{B}	9.3 ± 1.1^{A}
15.N-*21*	1	41	41^{B}	11.8 ± 0.8	3	78	0^{A}	N/A
	2	28	57^{BC}	13.6 ± 0.8^{AB}	4	53	6^{AB}	7.6 ± 2.1
	Both	69	48^{B}	12.6 ± 0.6^{AB}	Both	131	2^{A}	7.7 ± 2.2^{A}
7_1	1	37	57^{B}	12.5 ± 0.9	3	94	15^{B}	22.3 ± 2.0^{B}
	2	132	70^{C}	15.3 ± 0.8^{AB}	4	105	2^{A}	14.5 ± 2.5
	Both	169	67^{C}	14.8 ± 0.7^{AB}	Both	199	8^{B}	21.3 ± 1.9^{B}

[a] Within each hatch, mean percent dead values with no common superscript letter differ ($P<0.01$).

[b] Within each hatch, mean days to death values with no common superscript letter differ ($P<0.01$). Analysis of variance showed the days to death for hatches 1 and 4 were not significantly different among lines. Therefore, no statistical comparisons of the mean days to death between lines for hatches 1 and 4 were conducted.

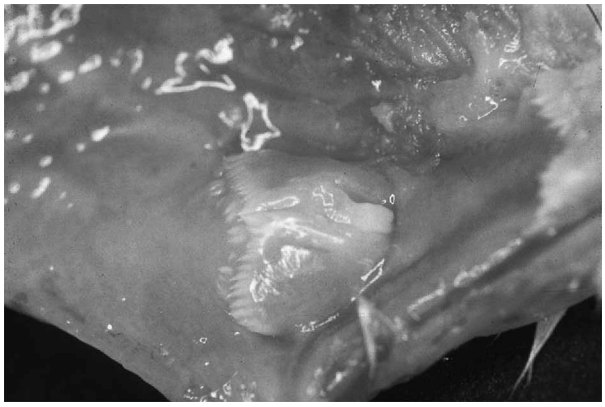

Figure 1. *Plug of mucus and exudate occluding the aditus larygnis of a 2-week-old chick infected with infectious bronchitis virus. Death was attributed to suffocation.*

and edema of the lamina propria, infiltration of the lamina propria with mononuclear cells and loss of epithelial cells into the tracheal lumen (Figures 3 and 4). The diagnosis of IB in these chicks was based on the gross and histological lesions compatible with IB and the isolation of corona virus from affected respiratory tissues (trachea, lung and air sac).

RNA from the isolates was not sequenced, so the IBV isolate was not defined. The isolates were not maintained. Death of the chickens was attributable to asphyxiation caused by occlusion of the trachea and larynx with mucus, fibrin and inflammatory debris. No attempt was made to isolate bacteria.

In contrast to hatches one and two, when chicks in hatches three and four received the appropriate

Figure 2. *Trachea of a 1-week-old chick infected with infectious bronchitis virus. The tracheal lumen is occluded with a plug of mucus and exudates.*

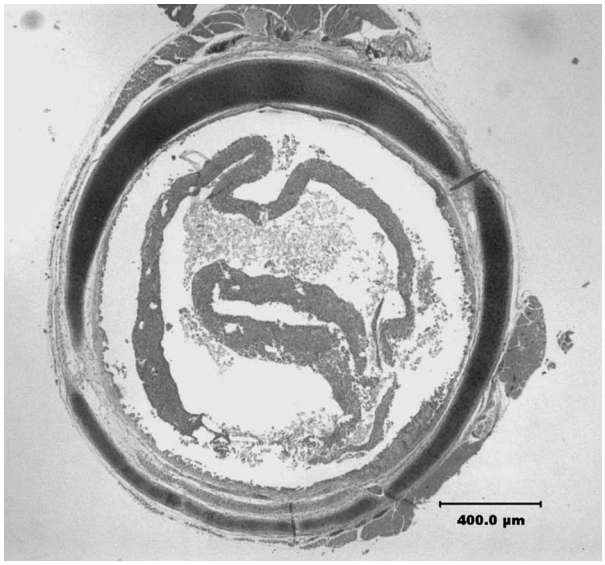

Figure 3. *Cross-section of the trachea of a 1-week-old chick infected with infectious bronchitis virus. There is loss of the epithelium, marked inflammation of the lamina propria, and exudation and epithelial sloughing into the lumen.*

IB vaccine only 0% to 10% of the chicks in these lines died.

Discussion

The ADOL inbred line $15I_5$ was resistant to the development of IB in contrast to inbred line 7_1 following inappropriate administration of a low attenuated IBV to 1-day-old chicks. The greater susceptibility to IB of 7_1 compared with $15I_5$ agrees with earlier results from England where 2-week-old chicks were inoculated with a pool of 10 strains of Massachusetts type IBV (Bumstead *et al*., 1989; Cook *et al*., 1990). Line 7_2 (an ADOL line homozygous for *B*2* related to 7_1; Bacon *et al*., 2000) was more susceptible to IB than line 15I

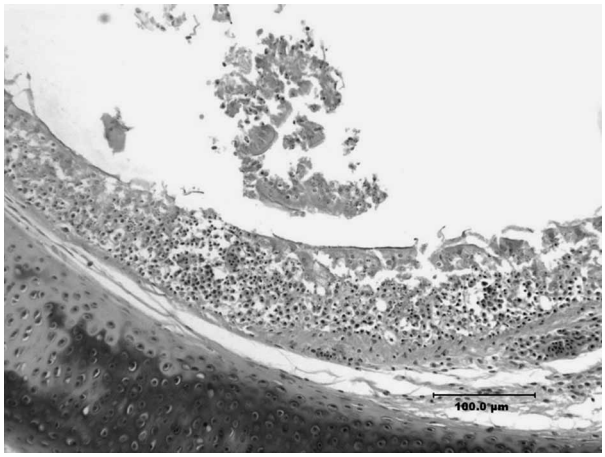

Figure 4. *Trachea of a 2-week-old chick infected with infectious bronchitis virus. There is edema, hyperemia and necrosis in the lamina propria and infiltration of mononuclear cells, many of which are necrotic. There is sloughing of the pseudostratified columnar epithelium into the lumen.*

(an ADOL line homozygous for *B*15* from which $15I_5$ was derived; Bacon *et. al*., 2000). However, line 15I developed considerable mortality compared with other more resistant lines (e.g. line C) or compared with $15I_5$ in this report. Moreover, when 2-week-old chicks were inoculated with a combination of the IBV pool and a pool of four strains of bacterenemic *Escherichia coli* of four different serotypes, lines 15I and 7_2 were both highly susceptible to IB (Bumstead *et al*., 1989). In contrast, line C and a Brown Leghorn line (BrL), remained resistant to IB. The data from lines C and BrL indicate that some chicken strains are more resistant to IBV than line $15I_5$. The line C used in England is also maintained and available at the ADOL (Bacon *et al*., 2000).

Following an intranasal inoculation of vaccine inappropriately at 1 day of age the chicks of line $15I_5$ with *B*15* were more resistant to IB than the chicks of B congenic lines 15.P-*13* (*B*13*) or 15.N-*21* (*B*21*) (Table 1). This was seen in two repeated hatches. This indicates that genes involving the *B* haplotype or tightly linked genes influence resistance to IB. We presume the variance in resistance is due to differences in antibody or cell-mediated immune responses to IBV. Resistance to IBV has not been studied previously with these or, to our knowledge, other B congenic lines, nor are we aware of other studies that have linked the *B* haplotype to differences in immunity to IBV. However, in England following an intranasal inoculation of a mixture of IBV strains and *E. coli* strains (see earlier) in 2-week-old F_2 or backcross chicks, there was no evidence that the MHC influenced the response to IBV (Bumstead *et al*., 1989). The chicks were from crosses of lines 7_2 (*B*2*) and BrL (*B*119* and *B*120*), or 15I (*B*15*) and C (*B*4* and *B*12*). Their data indicated that strong non-MHC genetic influences exist for resistance to IBV. Their failure to detect a MHC influence on IBV contrasts with the present data, but the difference may be attributable to differences in the IBV, inclusion of *E. coli*, age of exposure, difference in *B* haplotypes, or other factors. To formally establish a linkage of the *B* haplotype with IBV resistance in the B congenic lines, F_2 chickens should be evaluated that segregate for the *B* haplotype. This was not achievable due to retirement of the senior author, the distance between the laboratories, and other research missions.

The MHC has been shown repeatedly to clearly influence resistance to MD (Bacon *et al*., 2001). Indeed, the ADOL 15.*B* congenic line 15.N-*21* is resistant to tumors induced by a mild MD virus in contrast to the other B congenic lines, including line $15I_5$. Moreover, chicks of the B congenic lines are shown to differ in development of vaccinal immunity when challenged with very virulent MD virus. Following vaccination some *B* congenic lines (e.g. $15I_5$) are protected just as well as 15.N-*21*, but some are less responsive (e.g. 15.P-*13*). Thus, the 15.*B* congenic lines have been used to show that genes linked to the B haplotype can have a marked influence on MD, and vaccinal immunity to MD. The current observations indicate these lines may also be useful in defining B haplotype-linked influences on resistance to viruses determining diseases of the respiratory system (e.g. IBV), as well as differences in response to vaccines to these viruses. In addition to the three 15.*B* congenic lines used here, five other 15.*B* congenic lines are available: two with *B2* (15.6-*2* or 15.7-*2*), *B5* (15.15I-*5*), *B12* from line C (15.C-*12*) and *B19* (15.P-*19*) (Bacon *et al*., 2000). Importantly, the DNA sequences for the predominantly expressed class I (*BF2*) and class II (*BL2*) genes in these *B* haplotypes are defined (see Miller *et al*., 2004).

Many strains of IBV have been described and may be useful for analyzing genetic resistance to IBV (Raj & Jones, 1997; Cavanagh & Naqi, 2003). However, as with MD virus, a study of genetic resistance to IBV in unvaccinated chickens will probably require a mild IBV strain that causes symptoms and perhaps mortality in susceptible chicken lines, but no mortality and/or signs of rapid recovery in resistant lines. As with MDV, it appears all lines of chickens can be infected with IBV, but they can differ in disease development. Experiments will also require infection at an optimal age, and may be more effective if chicks lack maternal antibody.

The chicken is an ideal animal model for studying vaccine-induced immunity and genetic resistance to coronaviruses (Cavanagh, 2003). Mechanisms of resistance to IBV in the chicken are of current interest because of the recent outbreak of SARS (severe acute respiratory syndrome), a coronavirus in humans that has genomic resemblance to IBV (Zeng *et al*., 2003). Very recently, an epitope was identified on the spike protein of the SARS virus that elicits specific immune responses mediated by cytotoxic T cells that is MHC restricted (Wang *et al*., 2004). It is probable that the definition of an array of comparable MHC-defined IBV epitopes determining resistance in the chicken could result in development of improved vaccines that incite better immune responses in chickens of many *B* haplotypes. Trials using *B*15* versus *B*13* or *B*21* chickens infected with an appropriate IBV isolate may result in the identification of the precise epitope on an IBV protein that incites the specific immune response. Both cell-mediated and humoral immune responses to IBV are now well defined in chickens and available to analyze the epitope/proteins (Cook *et al*., 1991; Pei *et al*., 2003). Indeed, the Collison Laboratory has concluded that cell-mediated immunity is dependent upon MHC compatibility between responder and target cells (Seo *et al.*, 1997; Pei *et al.*, 2003). Thus, resistance that is

attributable to the *B* haplotype may result in the identification of immunogenic epitopes. Alternatively, the current data indicate that one could select for chickens with the *B*15* haplotype to increase resistance to IBV. Indeed, the resistant *B*15* haplotype is prevalent in both egg-laying and broiler chicken strains (Briles & Briles, 1982; Li *et al*., 1999). However, it is unlikely selection for a resistant *B* haplotype (e.g. *B*15*) would be desirable. This could be counter-productive since chickens must have immune responsiveness to a variety of pathogens as well as other strains of IBV (which could recombine), some of which may be susceptible in the *B*15* haplotype.

We have described retrospective evidence that the *B* haplotype has an influence on IB in chickens from two replicate hatches. It is hoped that these retrospective data will encourage laboratories with a mission to define resistance to respiratory viruses to explore evidence for genetic, and especially *B* haplotype influences on resistance to IBV.

Acknowledgements

This work was conducted in 1997 during a sabbatical study by Larry Bacon at the University of Guelph. The authors thank Ms Kathy Buttrum for assistance in egg incubation, hatching and attentive care of chicks. The study was supported in part by the Natural Sciences and Engineering Research Council of Canada and the Ontario Ministry of Agriculture, Food and Rural Affairs. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

References

Bacon, L.D., Hunt, H.D. & Cheng, H.H. (2000). A review of the development of chicken lines to resolve genes determining resistance to diseases. *Poultry Science*, *79*, 1082–1093.

Bacon, L.D., Hunt, H.D. & Cheng, H.H. (2001). Genetic resistance to Marek's Disease. In K. Hirai (Ed.), *Current Topics in Microbiology and Immunology, Vol. 225 Marek's Disease* (pp. 121–137). Berlin: Springer-Verlag.

Briles, E.W. & Briles, R. (1982). Identification of haplotypes of the chicken major histocompatibility complex (B). *Immunogenetics*, *15*, 449–459.

Bumstead, N., Huggins, M.B. & Cook, J.K.A. (1989). Genetic differences in susceptibility to a mixture of avian infectious bronchitis virus and *Escherichia coli*. *British Poultry Science*, *30*, 39–48.

Cavanagh, D. (2003). Severe acute respiratory syndrome vaccine development: experiences of vaccination against avian infectious bronchitis coronavirus. *Avian Pathology*, *32*, 567–582.

Cavanagh, D. & Naqi, S.A. (2003). Infectious bronchitis. In Y.M. Saif, A.M. Fadly, L.R. McDougle & D.E. Swayne (Eds.), *Diseases of Poultry* 11th edn (pp. 101–119). Ames: Iowa State Press.

Cook, J., Otsuki, K., Huggins, M. & Bumstead, N. (1990). Investigations into resistance of chicken lines to infection with infectious bronchitis virus. *Advances in Experimental Medicine and Biology*, *276*, 491–496.

Cook, J.K., Davison, T.F., Huggins, M.B. & McLaughlan, P. (1991). Effect of in ovo bursectomy on the course of an infectious bronchitis virus infection in line C White Leghorn chickens. *Archives of Virology*, *118*, 225–234.

Geng, S. & Hills, F.J. (1989). *Biometrics in Agricultural Science* (pp. 215–217). Dubuque, Iowa: Kendall/Hunt Publishing.

Li, L., Johnson, L.W., Livant, E.J. & Ewald, S.J. (1999). The MHC of a broiler chicken line: serology, B-G genotypes, and B-F/B-LB sequences. *Immunogenetics*, *49*, 215–224.

Miller, M.M., Bacon, L.D., Hunt, H.D., Ewald, S.J., Kaufman, J., Zoorob, R. & Briles, W.E. (2004). 2004 nomenclature for the chicken major histocompatibility (B and Y) complex, *Immunogenetics, 56*, 261–279.

Pei, J., Briles, W.E. & Collisson, E.W. (2003). Memory T cells protect chicks from acute infectious bronchitis virus infection. *Virology*, *306*, 376–384.

Raj, G.D. & Jones, R.C. (1997). Infectious bronchitis virus: immunopathogenesis of infection in the chicken. *Avian Pathology*, *26*, 677–706.

SAS Institute (2001). *SAS Version 8.02*. Cary, North Carolina: SAS Institute.

Seo, S.H., Wang, L., Smith, R. & Collison, E.W. (1997). The carboxyl-terminal 120-residue polypeptide of infectious bronchitis virus necleocapsid induces cytotoxic T lymphocytes and protects chickens from acute infection. *Journal of Virology*, *71*, 7889–7894.

Wang, B., Chen, H., Jiang, X., Zhang, M., Wan, T., Li, N., Zhou, X., Wu, Y., Yang, F., Yu, Y., Wang, X., Yang, R. & Cao, X. (2004). Identification of an HLA-A*0201-restricted $CD8^+$T-cell epitope SSp-1 of SARS-CoV spike protein. *Blood*, *204*, 200–206.

Zeng, F.Y., Chan, C.M.W., Chan, M.N., Chen, J.D., Chow, K.Y.C., Hon, C.C., Hui, K.H., Li, J., Li, V.Y.Y., Wang, C.Y., Wang, P.Y., Guan, Y., Zheng, B., Poon, L.L.M, Chan, K.H., Yuen, K.Y., Peiris, J.S.M. & Leung, F.C. (2003). The complete genome sequence of severe acute respiratory syndrome coronavirus strain HKU-39849 (HK-39). *Society for Experimental Biology and Medicine*, *228*, 866–873.